Polyacrylamide gel electrophoresis was performed in a vertical cell, using Tris-EDTA-borate buffer at pH 8.6. The gel was prepared with an acrylamide concentration of 7 % and a urea molarity of 4.5. The protein bands were stained with Amido Black solution.

Results and Discussion

Quantitative data for all experiments are presented in Table 1. Different milk samples contained variable amounts of soluble proteins. This was observed also in previous research (1).

The electrophoretic patterns of the protein fractions are shown in Fig. 1. Some of the zones could not be well reproduced in photographic prints. The main protein components were identified by using micelles and whey proteins as standards. Micelles were prepared from skim milk by ultracentrifugation at 78,500 g for 60 minutes at 25 ° C. Whey proteins were prepared from skim milk after the caseins were precipitated at pH 4.6. The whey was dialyzed against distilled water for three days, then the nondialyzable fraction was freeze-dried.

Chloroform used alone as solvent (Experiment II) extracted only lipids from the milk. When methanol was used alone (3 volumes and 20 volumes of methanol to 1 volume of milk) α-lactalbumin remained soluble and sometimes also trace amounts of other minor proteins, but γ-casein and temperature-sensitive casein were not soluble. However, when the chloroform-methanol mixture was used, many minor proteins of milk remained soluble, while α- and β-caseins and whey proteins were precipitated.

Electrophoresis showed that the composition of soluble protein fractions correlated with the ratio of fluid whole milk to chloroform-methanol, Fig. 1 (2 and 3). In ratios 1:15 and 1:20 the mixture formed only one liquid phase and the soluble protein fraction contained γ-casein, temperature-sensitive casein and other minor components, Fig. 1 (2). This composition was qualitatively identical to that of the CMEP fraction obtained from acid-precipitated casein (1) or dried milk powder or casein ultracentrifugate.

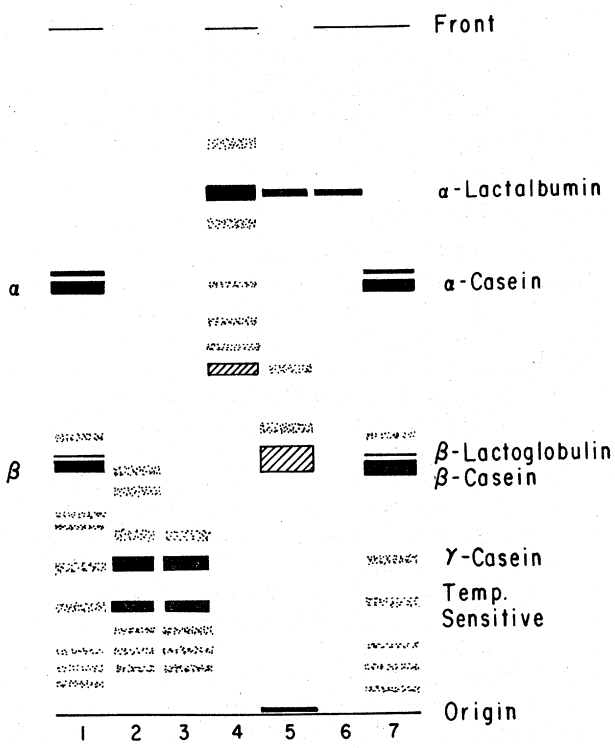

Fig. 1. Polyacrylamide gel electrophoresis patterns of milk protein fractions. 1) and 7) Casein micelles; 2) Proteins (CMEP) of one-phase extract (1:15, 1:20); 3) Proteins (CMEP) of chloroform layer of the two-phase extracts (1:5, 1:8, 1:10); 4) Proteins (MSP) of methanol layer of the two-phase extracts (1:5, 1:8, 1:10); 5) Whey proteins; 6) Proteins obtained with 20 volumes methanol. (The composition of proteins obtained with 3 volumes of methanol was identical with that obtained with 20 volumes of methanol.)

With ratios of 1:5, 1:8, and 1:10 of milk to chloroform-methanol the mixture formed a two-phase liquid system, chloroform layer and aqueous layer. The chloroform layer contained γ-casein, temperature-sensitive casein and other minor components. The composition was identical to the CMEP fraction obtained in experiments with ratios 1:15 and 1:20, Fig. 1 (2),

Table 1. Yield of extractable proteins from milk and from acid precipitated casein

	Ratio milk : solvent	Total extractable proteins g/l fresh milk	Remarks
Commercial homogenized milk extracted with $CHCl_3$-MeOH	1 : 5	1.1	2 liquid phases
"	1 : 8	1.5	"
"	1 : 8	2.8	"
"	1 : 10	1.6	"
"	1 : 10	2.3	"
"	1 : 10	2.4	"
"	1 : 10	2.2	" [a]
"	1 : 15	0.8	1 liquid phase
"	1 : 20	0.5	"
"	1 : 20	1.7	"
Fresh whole milk extracted with $CHCl_3$-MeOH	1 : 8	3.3	2 liquid phases
"	1 : 8	2.1	"
"	1 : 10	2.0	"
" (2)	1 : 10	2.0—2.5	"
Acid precipitated casein from fresh milk (1) extracted with $CHCl_3$-MeOH		0.6	1 liquid phase
Acid precipitated casein from comm. homog. milk (1) extracted with $CHCl_3$-MeOH		0.15	"
Fresh whole milk extracted with chloroform	1 : 10	0.0	2 liquid phases
Commercial homog. milk extracted with methanol	1 : 3	1.6	1 liquid phase
Commercial homog. milk extracted with methanol	1 : 20	0.16	"

[a] Mixture was stirred for 5 minutes only.

and the CMEP fraction obtained from acid precipitated casein (1).

The aqueous methanol phase protein fraction contained none of the chloroform phase proteins (CMEP) but did contain others, Fig. 1 (4). The composition of the aqueous-methanol phase fraction was not identical to that of the protein fraction obtained directly with three volumes and 20 volumes of methanol to one volume of milk, Fig. 1 (6). These proteins appeared in solution only when the chloroform-methanol solvent system formed two liquid phases with whole milk (ratios 1:5, 1:8, 1:10). I propose to call this fraction the methanol soluble proteins (MSP).

Extraction of coagulated proteins (Experiment IV), obtained with chloroform-methanol, gave a protein fraction electrophoretically identical to that obtained from the acid precipitated casein (1) and from whole milk experiments with milk to solvent ratios 1:15 and 1:20, Fig. 1 (2).

When the chloroform phase was evaporated to dryness, the residual proteins were no longer soluble in chloroform. This suggests that the CMEP originally in milk may have been there as lipid-protein complexes but on evaporation of solvent the complexes were denatured and broken down to lipids and proteins. These conjugated protein and lipid molecules exhibiting the solubility properties of the lipids rather than the protein moiety were classified by FOLCH et al. (4—7) as proteolipids.

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Zusammenfassung

Cerbulis, J.: **Selektive Abtrennung einiger Proteine aus Milch mit Hilfe von Fettlösungsmitteln.** *„Milchwissenschaft"* **25.** *(2) 76—78 (1970).*

24 Eiweißanalyse (Fettlösungsmittel).

Die Menge und Zusammensetzung der in Chloroform-Methanol löslichen Proteine in Vollmilch korrelieren mit dem Verhältnis Milch/Chloroform-Methanol. Mit einem Chloroform/Milch-Verhältnis von 1:15 und 1:20 wurden einphasige Lösungen erhalten; die Zusammensetzung der löslichen Proteinfraktion (CMEP γ-caseinhaltig), temperaturempfindliches Casein und weitere Minorproteine in Milch) war mit der mit Hilfe von Chloroform-Methanol extrahierbaren Proteinfraktion identisch, die aus säuregefälltem Casein oder aus Caseinmizellen gewonnen wurde. Bei einem niedrigeren Verhältnis (1:5 bis 1:10) von Milch zu Chloroform-Methanol bildete letzteres zwei flüssige Phasen, und zwar die Chloroform- und die wässerige Methanolphase. Diese Lösungen zusammen erbrachten größere Mengen an löslichen Proteinen; die Zusammensetzung war komplexer als von einphasigen Lösungen (Verhältnis 1:15 und 1:20). Die Zusammensetzung der Proteine der Chloroformphase war elektrophoretisch mit CMEP identisch, die aus säuregefälltem Casein oder Caseinmizellen erhalten wurde. Die wässerige Methanolphase enthielt andere Proteine als die Chloroformphase (CMEP). Die in der wässerigen Methanolphase vorhandenen Proteine werden „methanollösliche Proteine" (MSP) genannt. Mit Chloroform allein konnten keine Proteine aus Vollmilch extrahiert werden. Dok.-Ref.

Cerbulis, J.: **Selective separation of some proteins from milk with fat solvents.** *„Milchwissenschaft"* **25.** *(2) 76—78 (1970.*

24 Protein analysis (fat solvents).

The amount and composition of the chloroform-methanol soluble proteins in whole milk correlate with the ratio of milk to chloroform-methanol. With ratios of 1:15 and 1:20 of milk to chloroform-methanol, one-phase solutions were obtained and the composition of the souble protein fraction (CMEP, containing γ-casein, temperature-sensitive casein and other minor proteins in milk) was identical with that of the chloroform-methanol, extractable protein fraction obtained from acid-precipitated casein

or casein micelles. Chloroform-methanol, with smaller ratios (1:5 to 1:10) of milk to chloroform-methanol, formed two liquid phases, chloroform and aqueous methanol phases. These solutions together yielded larger amounts of soluble proteins and the composition was more complex than from one-phase solutions (ratios 1:15 and 1:20). The composition of the chloroform-phase proteins was electrophoretically identical to CMEP obtained from acid-precipitated casein or casein micelles. The aqueous methanol phase contained other proteins than the proteins in the chloroform phase (CMEP). The proteins present in the aqueous methanol phase are called methanol soluble proteins (MSP). Chloroform alone did not extract proteins from whole milk.

CERBULIS, J.: **Séparation sélective de quelques protéines du lait en utilisant des agents liposolubles.** *„Milchwissenschaft“* **25.** *(2) 76—78 (1970).*

24 Analyse de protéines (agents liposolubles).

CERBULIS, J.: **Separación selectiva de algunas proteinas de la leche mediante agentes liposolubles.** *„Milchwissenschaft“* **25.** *(2) 76—78 (1970).*

24 Análisis de proteinas (agentes liposolubles).

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Selective separation of some proteins from milk with fat solvents

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Introduction

A previous report (1) showed that acid-precipitated casein by extraction with chloroform-methanol (2:1) yielded 0.6 g chloroform-methanol extractable proteins (CMEP) per liter of pooled milk, equivalent to 2 % of total protein. However, by the treatment of whole milk with chloroform-methanol (1 volume of milk to 10 of solvent), approximately 2.2 g of CMEP were obtained from 1 liter of pooled milk (equivalent of 6 % to total protein) (2). Also, the composition of the CMEP by the two methods was different.

The treatment of whole milk with chloroform-methanol has now been studied further to explain the differences in CMEP yield and composition.

Methods

Fresh pooled cow milk (fat content approximately 4.5 %) and commercial homogenized pasteurized milk (fat content 3.3 %) were used for the following experiments:

I. Chloroform-methanol (2:1, v/v) was added to milk and the mixture was vigorously stirred for 3 hours, then filtered. The ratios of milk to chloroform-methanol were 1:5, 1:8, 1:10, 1:15, and 1:20. In all experiments precipitated proteins were present in the mixture.

A complex system was obtained with milk to solvent ratios of 1:5, 1:8, and 1:10. It consisted of an aqueous methanol layer on top, coagulated proteins, and a chloroform layer. After filtration, the aqueous methanol layer and the chloroform layer were separated and each layer was analyzed separately. The chloroform layer was evaporated on a steam bath with a stream of nitrogen. The residue was extracted with petroleum ether and the insoluble residue was analyzed by electrophoresis (1). The aqueous methanol layer was dialyzed against distilled water for 3 days and the nondialyzable fraction was freeze-dried, then ex-

tracted with petroleum ether and the petroleum ether insoluble fraction (proteins) was analyzed by electrophoresis (1).

With ratios of milk to solvent of 1:15 and 1:20, one liquid phase and coagulated proteins were obtained. After filtration the filtrate was evaporated to dryness on a steam bath with a stream of nitrogen. The residue was taken up with 200 ml chloroform-methanol (2:1) and 40 ml. 0.1 M KCl was added, as described by FOLCH et al. (3). Aqueous methanol, interphase solids, and chloroform layers were obtained. The chloroform layer (lipids) was separated and washed once with 0.1 M KCl, and the chloroform fraction was discarded. The washing was added to the aqueous methanol phase with interphase solids and dialyzed against distilled water for 3 days. The nondialyzable fraction was freeze-dried, extracted once with petroleum ether, and the protein fraction was analyzed by electrophoresis (1).

II. Ten volumes of chloroform were added to one volume of milk and the mixture was vigorously stirred for 3 hours, then filtered. The filtrate was evaporated to dryness and the residue was taken up with petroleum ether. No petroleum ether-insoluble material (proteins) was present in the extract.

III. Three volumes and 20 volumes of methanol were added to one volume of milk samples and the mixtures were stirred for 3 hours and filtered. The filtrates were concentrated to approximately 200 ml in vacuo, then dialyzed for 3 days and the nondialyzable fraction was freeze-dried. The residues were extracted with petroleum ether and the petroleum ether insoluble fractions (proteins) were studied by electrophoresis (1).

IV. The coagulated proteins obtained from the chloroform-methanol experiments described above were extracted with chloroform-methanol (2:1), as described for acid-precipitated casein (1), and the extracts treated, as described in Experiment I, for extracts obtained with milk-to-solvent ratios of 1:15 and 1:20.